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Running title: Rainbow trout resistance and tolerance

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Abstract

Parasite infectivity, virulence and host resistance have been in the centre of the scientific interest when it comes to host-parasite relationships. In addition to resistance, hosts may also vary in their tolerance against parasites. This is important to notice because resistance and tolerance have different consequences in host-parasite coevolution. Here we show that families of farmed rainbow trout (Oncorhynchus mykiss) show both host defence-strategies, resistance, and tolerance, against infectivity and virulence of Diplostomum sp. (Trematoda) parasites. Both strategies have moderate genetic variation and are genetically independent of each other. It is also shown that the families having the highest performance measured as higher weight, better condition factor and lower mortality in absence of the parasites, suffer the most when parasitism increases. For practical breeding programmes, this means that both resistance, and tolerance, can be improved by selection without compromising one of the strategies. These results give new insight into defence strategies against parasites in fish and into processes of fish-parasite coevolution.

Key words: rainbow trout, Diplostomum, resistance, tolerance, host-parasite relationship, breeding programme

1. Introduction

Parasites are considered better competitors than their hosts. This is because parasites have shorter generation times, higher migration potential and thus good abilities to overcome host resistance (Ebert & Hamilton, 1996). Therefore, parasites are often in centre of interest in the research of host-parasite coevolution. Understanding how parasites are adapted to exploit their hosts remains a central question in the evolutionary ecology of host–parasite interactions (Alizon, Hurford, Mideo, & Van Baalen, 2009; Anderson & May, 1982; Frank, 1996). On the host side, not only resistance but also tolerance against parasites affects host fitness. Resistance refers to the mechanisms that reduce parasite burden, whereas tolerance refers to the mechanism that minimise fitness impact of parasites on their host (Svensson & Råberg, 2010). Host tolerance and resistance have totally different evolutionary implications and practical consequences in terms of host-parasite relationships (Hayward et al., 2014; Råberg, Sim, & Read, 2007). Host resistance challenges parasites to evolve their infectivity, whereas host tolerance does not directly limit parasite success (Svensson & Råberg, 2010). Resistance is an extensively studied topic, but empirical studies on host tolerance against parasites are still quite rare (but see Balard et al., 2020; Kutzer & Armitage, 2016).

Diplostomum sp. (Trematoda) are common parasites of several species in fresh and brackish water aquaculture in Finland (Valtonen, Hakalahti-Sirén, Karvonen, & Pulkkinen, 2012). Fishes, like rainbow trout are a secondary host for this parasite (Palmieri, Heckmann, & Evans, 1976). Sexual reproduction of Diplostomum sp. takes place in the intestine of a piscivorous bird, like seagull (Larus spp.) (Crowden & Broom, 1980). Parasite eggs are then released to water via bird faeces, hatched miracidia larvae infect snails (Lymnaea spp.), reproduce asexually and cercaria are again released in water where they infect bypassing fish (Crowden & Broom, 1980). Diplostomum sp. cercaria penetrate fish skin, gills or eyes and find their way to the eye lens (Palmieri et al., 1976). These parasites harm fish hosts by destroying eye lenses and vision. They reduce fish escape behaviour, and infected fish are easily predated by seagulls (Seppälä, Karvonen, & Valtonen, 2004). Costs of this parasite are also seen as reduced feeding ability (Crowden & Broom, 1980) and decreased fish growth in food production (Kuukka-Anttila, Peuhkuri, Kolari, Paananen, & Kause, 2010). This parasite is only shortly exposed to fish resistance before reaching eye lens, where parasites are out of reach of fish immune system. Diplostomum sp. tolerance would mean limited impact of the parasite on host performance and fitness.

A prerequisite for evolution of resistance or tolerance within populations is the existence of genetic variation in these traits. Genetic variance in fish resistance against Diplostomum sp. has been documented (Kuukka-Anttila et al., 2010) but there are no studies on genetic variance in fish tolerance, from a viewpoint of fish performance, against this parasite. However, a study on Atlantic salmon (Salmo salar) and sea trout (Salmo trutta trutta) showed that a between-species difference exists both in resistance and tolerance against Diplostomum sp. but a between-population difference only in resistance (Klemme & Karvonen, 2017).

We studied genetics of resistance and tolerance in a farmed rainbow trout (Oncorhynchus mykiss) population exposed to natural parasitic infections of Diplostomum sp. The objective was 1) to estimate genetic variation in host resistance and tolerance against parasite infection, 2) to assess whether resistance and tolerance are two genetically different defence-strategies of the host and, 3) in order to find any trade-offs for resistance or tolerance that could interfere the evolution of fish defence-mechanisms, to estimate genetic correlations of resistance and tolerance with host growth, condition, and survival.

2. Material and Methods

2.1. Study populations

Rainbow trout originated from the selection programme maintained by Natural Resources Institute Finland (Luke) at Tervo Aquaculture station in central Finland. The population was established in the late 1980s, and fish have been selected for growth, age of maturity, skin and flesh colour, survival, healthy eyes and body shape (Kause, Ritola, Paananen, Wahlroos, & Mäntysaari, 2005). Selection for healthy eyes started in 2003. In the breeding programme, the eyes are visually scored with 0 = both eyes healthy, 1 = one eye with cataract, and 2 = both eyes with cataract. Tervo Aquaculture station gets incoming water from a freshwater stream and Diplostomum sp. cercaria occur naturally in the water. Cercaria are released from snails in the stream during summer months, when water temperature is above 10 ˚C (Hakalahti, Karvonen, & Valtonen, 2006; Karvonen, Seppälä, & Valtonen, 2004). These host and parasite populations have coexisted since late 1980s when the breeding programme was established at the farm.

2.2. Mating design and rearing procedure

To produce the experimental offspring generation, broodstock fish were mated in a partial factorial mating design, where each sire was mated on average with 1.39 dams and each dam was mated on average with 1.35 sires (Table 1). To generate a total of 50 families, fertilizations with 36 sires and 37 dams were completed within two days in April 2005. Eggs were incubated in a single incubator, and family identification was ensured by dividing incubation trays into subsections for each family. At the eyed-egg stage in June 2005, fish were transferred to 150-l family-specific indoor tanks.

At an average weight of 103 g, in February 2006 (later called “juvenile stage”), fish were individually tagged using passive integrated transponders (Trovan Ltd., Munich, Germany) and measured for weight and length (traits: Weight1, Length1). The eyes of these 1498 fish (29-31 fish from each family) were examined with KOWA SL-15 slit-lamp microscope (Kowa Company, Ltd., Tokyo, Japan). The number of the parasites in each lens was counted. Slit-lamp microscope gives a clear three-dimensional view of the interior of a lens. When parasite number is low, it is easy to count them one by one. Cataract coverage in each eye-lens was categorised on scale 0-4 (0=no cataract, 1 = < 10 % coverage, 2 = 10-50 % coverage, 3 = 50-75 % coverage, 4 = 75-100 % coverage) according to categorisation by Wall and Bjerkås (1999). The number of parasites and the cataract scores from both eyes were summed to single values for parasite count (trait: Diplo1) and cataract score (trait: Cataract1), respectively, for each individual. Of the 1498 individuals, 357 had completely healthy eyes.

Parasite numbers can be also assessed via dissection of an eye lens (Klemme & Karvonen 2017; Scharsack & Kalbe, 2014). The reason why we did not use dissection is that by using slit-lamp, we could keep the fish alive for further growing and reduce unnecessary animal killing. Karvonen, Hudson, Seppälä, and Valtonen (2004) validated slit-lamp method and showed that a slit-lamp examination gives a reasonable estimate of parasite load. In our study, potential measurement error in parasite count results in increased residual variation for both resistance and tolerance, and hence the heritability and genetic variance estimates would be conservative and underestimates.

Half of the id-tagged fish from each family was left to be the parents of the next generation at the nucleus, and half were sent to a commercial farm to be recorded for further traits in the experiment. Due to visually based sorting during tagging, the fish left to the nucleus were slightly heavier (110.4 g) than the sea-tested fish (97.2 g). In addition, an average of 82.6 (range 46-104) fish per family (with average body weight of 70.7 g) were left untagged and discarded.

The discarded untagged fish within a family were weighed as a group and counted. Then, for each family, it was assumed that Weight1 has a normal distribution with a mean value calculated from the data. Standard deviation of a family was calculated as two times the standard deviation of the unculled observations that were above the family mean. For each family, individual Weight1 records for the culled individuals were then randomly drawn from the left-hand side of the normal distribution, below the quantile defined by the proportion of culled individuals, while simultaneously maintaining the original mean Weight1 of the culled group. These individual Weight1 records were added to the data. This practice generated normally distributed data for each family, with a correct family mean.

It is common practice in animal breeding programmes that selection occurs both across and within generations, to maximize genetic gain in the nucleus. If the non-random phenotypic recording is not accounted for in a statistical analysis, the estimated variances may become downward biased (Ouweltjes, Schaeffer, & Kennedy, 1988). In our genetic analysis, Weight1 was included as a trait into the analysis when estimating variances for all traits. In this way, the DMU software based on restricted maximum likelihood (Madsen, Sorensen, Su, Thomsen, & Labouriau, 2006) corrects for the non-random sampling. Simulations have shown that this practice prevents selection bias, i.e. restores trait variances to the level without the selection (Ouweltjes et al., 1988).

The fish were transferred to a commercial sea station in SW Finland in April 2006. They were reared in a single net pen and managed following the commercial practices of the farm. Throughout the cultivation, the fish were fed ad libitum with commercial pellet feed (Raisio group, Raisio, Finland). In December 2006, after the growing season (later called "1.5-year stage"), the fish were measured again for traits: weight (Weight2), length (Lenght2), and mortality (Mortality1>2) (Table 1). Mortality1>2 is the mortality between the first and the second measurements (1 = dead, individual tag not recorded, and 0 = live). Maturity state was scored visually into three categories based on the gonads of gutted fish: immature male, mature male, or immature fish. The females are all still immature at that age.

Condition factor of the fish (traits: Condition1, Condition2; Table 1) was calculated as residuals of the II type regression between weight and length at each recording time (Green, 2001).

2.3. Resistance and tolerance

As a measure of resistance, we used the number of parasites in the eye-lenses of the juvenile fish (Diplo1; Table 1). Tolerance was quantified as the slope of the regressions between fish performance (Weight1,2, Condition1,2, Mortality1>2) on y-axis and increasing parasite count (Diplo1) on x-axis. These performance traits were chosen because it was known that Diplostomum sp. causes reduced weight gain and condition (Crowden & Broom, 1980; Kuukka-Anttila et al., 2010). Measured in this way, tolerance quantifies the ability of a host to limit the impact of a given parasite burden on host performance (Kause, 2011; Simms, 2000). We used Diplo1, rather than Cataract1, on the x-axis because Diplo1 is the ultimate reason for reduction in fish performance. There was no visual indication of other pathogens in this study. No virological or bacteriological testing were, however, done.

2.4. Genetic analyses

Phenotypic and genetic variances and correlations were estimated using the DMU software (Madsen et al., 2006). The software uses multivariate mixed models with the restricted maximum likelihood

method, and accounts for all the relationships between all animals in the pedigree using a relationship matrix. The pedigree had a total of 355 ancestors without phenotypes in 5 generations as well as the offspring generation recorded for phenotypes.

The statistical 'trait mean' models to estimate (co)variance components for the traits: Weight1, Condition1, Diplo1, and Cataract1 recorded at juvenile stage were:

$y = \mu_i + animal_j + famtank_k + error$
$y = \mu_i + animal_j + error$

and the 'mean models' for the traits of Weight2, Condition2, and Mortality1>2 recorded at 1.5-year stage were:

$y = sexmat_l + animal_j + famtank_k + error$
$y = sexmat_l + animal_j + error$

where μ is the mean for a trait i, animal is the random genetic effect of an animal connected to the pedigree (j = 1…number of individuals), famtank is the random full sibling and family tank effect (without a link to a pedigree) to quantify the environmental effect common to full siblings (k = 1-50 tanks), sexmat is the fixed effect of gender and maturity stage (l = 1-3; immature male, mature male, immature fish). Error is the random error term.

Heritability (h^2) was calculated as the genetic variance explained by the animal effect divided by phenotypic variance (V_P), where V_P is the sum of genetic (V_G), common environment variance (V_C) and residual variance (V_R). Heritability was considered significantly different from zero when h^2 estimate - 0.98 SE did not include zero (one-tailed hypothesis). Genetic correlation (r_G) was considered smaller or greater than zero when r_G estimate +/- 1.96 SE did not include zero (two-tailed hypothesis). When family tank effect is included in the statistical model, the heritability estimate is 'narrow sense'. When family tank effect is excluded from the statistical model, the heritability estimate is termed 'broad-sense'.

The results from the 'mean model' for Diplo1 are the results for resistance.

To proceed to tolerance, a random regression model was applied in which the genetic variation in the slope of the regression quantifies the genetic variation in tolerance (Kause, 2011). For tolerance of performance traits (Weight1,2, Condition1,2, and Mortality1>2) against Diplo1, the random regression model was:

$y = sexmat_l + b_0 + b_1 + b_o\ anim_i + b_1\ anim_i + error,$

where b_0 is the fixed intercept of a regression, b_1 is the fixed regression slope of a fish performance trait (on y-axis) against Diplo1 (on x-axis), $b_0\ anim_i$ is the random genetic intercept effect, and $b_1\ anim_i$ is the random genetic slope effect of the regression. For Weight2, a model in which Weight1 was included as a fixed covariate was also run. In such a model, the fish trait analysed is weight gain and the initial differences in body weight are statistically accounted for.

Tolerance slope of an individual is not recorded in our data, and there is no phenotypic variation for the slope. Hence, heritability cannot be calculated for the tolerance slope in these random regressions, but the genetic variance in the slope can be directly used to quantify genetic variation in tolerance (Kause, 2011). Variance was considered significant when variance - 0.98 SE did not include zero (one-tailed hypothesis).

To estimate genetic correlations, family tank was included for Weight1 whereas for the other traits the models excluded the family tank effect. For Condition2, a model with Weight2 as a fixed covariate was also run. This was an approach to remove the impact of body weight on condition factor on genetic parameter estimation in the genetic analysis.

In the tolerance analysis, a visual examination revealed that the residuals were normally distributed and homogenous along the x-axis, and the phenotypic relationships were linear for the traits recorded at a continuous scale. In contrast, mortality is a binary trait, and hence the results of Mortality1>2 should be considered as approximations. It is well known that phenotypic relationships of the binary traits, calculated using a multivariate mixed model like in the present study, are downward biased, but reassuringly, the genetic relationship are unbiased (Mäntysaari, Quaas & Gröhn, 1991). Genetic results of a tolerance analysis with mortality data are hence expected to be robust.

3. Results

3.1. Resistance: Genetic variation

The broad sense heritabilities were high for Condition1 and 2, Weight1 and Weight2 (range in h^2 = 0.45-0.79) when family tank was not included in the model (Table 2). When the model included family tank, the heritability of Weigh1 was reduced from 0.59 to 0.25 and heritability of Weight2 from 0.45 to 0.23, as the tank effect explained 23 and 13 % of the variation. For Condition2, the heritability was reduced to zero when family tank was included in the model. This is unlikely a true situation but rather results from artificial confounding of genetic and family tank effects during the estimation process. During the first months, each full-sib family is in a single tank. In our data, each sire and dam have sibs, and more remote relatives, with offspring but it is typical that in some cases the pedigree is not effective in partitioning the environmental effect common to full sibs from the genetic effect of the full sibs (Gjerde et al., 2004). For the other traits, the tank effect explained minor proportion of the phenotypic variation without consequent changes in heritability estimates compared to the model without the tank effect.

Heritabilities for the resistance trait Diplo1, as well as for Cataract1 and for Mortality1>2 were of moderate degree in models either including or excluding the tank effect (range in h^2 = 0.14-0.29; Table 2). For these traits, the results indicate genetic variation that cannot be explained by non-genetic full sibling and common environmental effects.

3.2. Resistance: Genetic correlations

There was significant positive genetic correlation between Diplo1 and Weight1 in the juvenile stage (Table 3). Genetic correlation between Diplo1 and Catarac1 was high. Moderate positive genetic correlations were found between Cataract1 and both, Weight1 and Condition1. The families with higher body weight and condition, had more parasites and more severe cataracts.

At 1.5-year stage, the genetic correlations between Diplo1 and weight or condition were not significant (Table 3). Similarly, the genetic correlations between Cataract1 and weight or condition factor were not statistically significant. The genetic correlation between Diplo1 and weight gain between the two measurement (genetic correlation estimated with a model in which Weight1 was used as a fixed covariate for Weight2) was, however, negative and statistically significant. The change

compared to the juvenile phase is obvious: The families that had better resistance grew faster from juvenile stage to 1.5-year stage.

A significant negative genetic correlation was found between mortality and fish weight at 1.5-year stage (Table 3). The lighter the family, the higher the mortality was. Interestingly, genetic correlation between Mortality1>2 and Condition1 was significantly positive. Mortality was higher in families having high Condition1.

3.3. Tolerance: Genetic variation

In all tolerance regressions, there was significant genetic variation in the intercepts (Table 4), standard error was always smaller than genetic variance (Vg_{int}). This simply means that there are differences between families in these traits also when there are no Diplostomum sp. flukes in their eye lenses.

Significant genetic variation was found also in tolerance slopes of Weight2 and Condition2 against Diplo1, i.e., there was variation between families in how they were capable to gain weight and good condition despite the parasites (Table 4). Interestingly, this was not seen in the early juvenile stage but only at the 1.5-year stage when the parasites had had time to influence the hosts.

For the population as a whole, the average fixed regression slope (b_1) against parasite count was +0.3980 ± 0.0601 (±SE) for Cataract1, +10.4 ± 0.52 for Weight1, +11.0 ± 0.18 for Condition1, -153 ± 9.23 for Weight2, -123 ± 5.75 for Condition2, and +0.0118 ± 0.018 for Mortality1>2.

3.4. Tolerance slope and intercept: Genetic correlations

Strong negative genetic correlations were found between the intercepts and the tolerance slopes in the regressions of Weight2, Condition2, and Mortality1>2 against the parasite count (Table 4). This indicates that the higher the weight, the better the condition, or the lower the mortality without parasites, the stronger is the negative effect of the increasing parasite load on the host. Overall, it seems that the families with better performance in the absence of parasites suffer relatively more from parasitism than the families with lower performance.

3.5. Resistance and tolerance: Genetic correlations

All genetic correlations between resistance and tolerance were non-significant in this study (Table 5), i.e., there seem to be no genetic relationship between resistance and tolerance (measured in terms of parasite effect on weight, condition factor, and mortality). For these traits, the results thus indicate that resistance and tolerance are genetically independent of each other.

4. Discussion

We found moderate genetic variation in rainbow trout resistance against Diplostomum sp. flukes as reported also in earlier studies (Kuukka-Anttila et al., 2010; Vehviläinen, Kause, Kuukka-Anttila, Koskinen, & Paananen, 2012). Moderate positive genetic correlations were observed between resistance, cataract coverage, weight, and condition at 8 months aged fish. This indicates that at genetic level, susceptibility to parasite infection, higher weight, and good condition are linked at the juvenile phase. Heavier fish have more parasites. Similar results have been reported also earlier in

rainbow trout (Kuukka-Anttila et al., 2010) and in Arctic charr (Kortet, Lautala, Kekäläinen, Taskinen, & Hirvonen, 2017). At 1.5-year stage, these genetic correlations, however, turned negative. The families that had lower resistance, and thus more parasites, had lower growth after the first months. We suggest that there might be different genetic strategies that can be expressed by two extremes, i) slow juvenile growth but lower parasitism, investment in resistance and ii) fast juvenile growth despite increased parasitism and potential investments in parasite tolerance

There was no indication of genetic differences in parasite tolerance at the juvenile stage of these fish. However, we did find genetic variation in the tolerance traits at the age of 1.5 years. At this age, there were differences between the families in weight and condition when the number of parasites increased in their lenses. Family-wide differences in tolerance did not occur until measurement 2 potentially because the parasites caught in the first summer in the freshwater farm had had enough time to reduce host vision and fish performance. Fish also lived their first 10 months in small groups and in tanks where feeding, even with impaired vision, was easier than in the net pens. The competition for feed is more limited in small groups and feed was available also in the bottom of the tanks. In other words, some families seemingly tolerated increased parasite burden better than the others and were capable to grow better despite the parasites.

Based on these results, it might be a good optional strategy for a host fish not to combat with parasites but to put the effort on tolerance, i.e., maximise fitness despite the parasites. In theory, this could mean allocating resources to growth and feeding or better skills to feed despite reduced vision, instead of resistance mechanisms. The existence of genetic variation in resistance and tolerance means evolutionary potential for a trait change and therefore a possibility to perform different defence strategies in response to pathogen pressure. Within animal populations, studies on host genetic variance in resistance are more numerous compared to studies on host genetic variation in tolerance. This is mainly because measuring of tolerance on individuals is difficult, and hence sophisticated statistical models such as random-regressions are needed (Kause, 2011). Some examples of quantitative genetic studies include tolerance of sheep against gut worm infections (Nematoda) (Hayward et al., 2014) and tolerance of pigs against Porcine Reproductive and Respiratory Syndrome (PRRS) virus (Lough et al., 2018). In fish, genetic variation in the amount of fin erosion caused by parasite Tracheliastes polycolpus (Crustacea: Copepoda) in Leuciscus burdigalensis has been shown by Blanchet, Rey, and Loot (2010). Bailey, Strepparava, Wahli, and Segner (2019) reported variation in brown trout relative kidney size due to Tetracapsuloides bryosalmonae (Myxozoa) infection. Some examples from other species include tolerance variation measured as severity of anemia and weight loss in mice infected with parasite Plasmodium falciparum (Protozoa) (Råberg et al., 2007) and variation in tolerance measured as relative number of offspring in fruit fly Drosophila melanogaster infected with bacteria Pseudomonas aeruginosa (Vincent & Sharp, 2014).

The basic theories of genetic polymorphism in the host defence against pathogens include Red queen dynamics (Van Valen, 1973). Spreading of a new resistance gene in a population diminishes pathogen burden, resistance becomes less beneficial and selection towards resistance diminishes until the parasite potentially evolves to overcome host resistance again. This leads to a cycle of counter-adaptations and potentially to increased parasite virulence in the sympatric host (Read, 1994). A new tolerance gene in a population, in contrast, increases tolerance and thus diminishes selection towards resistance (Råberg et al., 2007). As a result, parasite burden increases and selection towards tolerance increases. However, this is only true, if the benefits of tolerance exceed its costs (Roy & Kirchner, 2000). Unlike resistance, tolerance of the host thus does not select for higher infectivity or virulence of the parasite. If not all the hosts attend the arms-race for increased resistance, but enhance tolerance instead, also parasites having lower infectivity or virulence may succeed. It can be hypothesised that host populations may affect parasite infectivity and host-parasite co-evolution by alternating resistance and tolerance strategies. The literature on regulation mechanisms in host-parasite relationships has focused on parasite feedback mechanisms to adjust infectivity (Anderson &

May, 1982; Frank, 1996). However, balancing mechanisms in both, host and parasite populations, may potentially act in concert. This could also explain the remaining variance of tolerance in wild populations (Blanchet et al., 2010), and, why old host–parasite associations tend to be less virulent (Read, 1994).

We found negative genetic correlation between the tolerance slope and the intercept in the all three tolerance regressions in the fish of 1.5 years of age. The high performance measured as higher weight, better condition factor, and lower mortality in the absence of parasites was genetically associated with strong reduction in performance when parasitism increased. This is an obvious genetic trade-off for tolerance, and such a mechanism may maintain genetic variation in tolerance, or at least slow down the erosion of the genetic variation. The sensitivity of high performing genotypes to increased parasitism conforms to the observation that in aquaculture species, the high performing genotypes are more sensitive to changes in diet, production environment, water temperature, fish density, and other environmental changes, compared to low performing genotypes (Sae-Lim et al., 2015). A quantitative review across 38 aquaculture species by Sae-Lim et al. (2015) found a median genetic correlation of -0.39 between tolerance slopes and intercepts.

Studies on plants have clearly shown that resistance and tolerance are two different components of plant defence with different effects on the fitness of both the plant and the enemy (Núñez-Farfán, Fornoni, & Valverde, 2007). Our result of non-existent genetic correlation between resistance and tolerance in the rainbow trout suggest that also in animals, resistance and tolerance may be two different, genetically independent host defence strategies and can simultaneously be expressed in an individual. This finding has important implications for our understanding of the epidemiology and the evolution of infectious diseases. Yet, it should be noted that our study has limited power to detect significant genetic correlations even though some of the estimates were moderate in magnitude (e.g., -0.37 in Table 5).

Our results show that in farmed rainbow trout, tolerance to Diplostomum sp. flukes could be improved genetically even without deteriorating resistance at the same time. This would have a positive effect on fish welfare and profitability of the commercial aquaculture operations, when Diplostomatidae are present at the fish farms. However, care should be taken not to breed solely for high growth performance as this can have detrimental impact on tolerance-related traits. The Finnish national breeding programme for rainbow trout currently has an easy-to-record cataract coverage in the selection index and has effective management manners to control Diplostomum sp. infections. The management practices include maintaining the bottom of raceways clean of solids and vegetation (in which snail hosts live) and by minimizing the access of seagulls, another parasite host, to any food sources at a farm. The current work provides the methodological and genetic basis for expanding the selection index to include also tolerance.

Ethical approval

The experiment was conducted according to the guidelines established by the Finnish Game and Fisheries Institute. Experiment was performed in accordance with the Finnish animal welfare legislation and comply with the directive 2010/63/EU implemented in Finnish legislation in the Act on the Use of Animals for Experimental Purposes (62/2006) (until 1.8.2013). All experimental fish were anesthetized with tricaine methanesulfonate before sampling to minimize suffering.

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Data Availability Statement

The data that support the findings of this study is available from Antti Kause upon request.

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Table 1. Data structure for studied traits. Number of fish recorded (n), trait means (mean), their standard deviations (sd) and range of variation (range).

Trait	n	mean	sd	range
Diplo1	1498	3.66	2.08	0-13
Cataract1	1498	3.44	1.11	0-7
Weight1	1497	103.84	21.16	47.7-213.2
Condition1	1497	1.03	8.62	-39.16-51.22
Weight2	638	1043.94	262.30	165-1895
Condition2	638	4.87	88.92	-207.07-638.00
Mortality1>2	751	0.148	0.36	0,1

Diplo = Diplostomum sp. parasite count in two eyes, Cataract = cataract coverage in two eyes, Weight = body mass, Condition = condition factor, Mortality = survival (0=alive, 1=dead). 1 refers to the first measurement (juvenile phase), 2 refers to the second measurement (1.5-year stage).

Table 2. Phenotypic variance (Vp), heritability (h^2 +/- SE standard error) and the tank effect (c^2 +/- SE standard error) in the measured traits estimated using a model either including or excluding random family tank effect. Trait abbreviations are explained in Table 1. Significant heritabilities are bolded and marked with *.

	No tank effect			Tank effect (c^2) included				
Trait	Vp	h^2	SE	Vp	h^2	SE	c^2	SE
Diplo1	1.136	0.29*	0.07	1.110	0.21*	0.11	0.03	0.04
Cataract1	0.3183	0.26*	0.06	0.3154	0.24*	0.10	0.005	0.03
Weight1	740.5	0.59*	0.09	725.0	0.25*	0.18	0.23	0.14
Condition1	96.46	0.79*	0.10	92.33	0.65*	0.18	0.04	0.05
Weight2	82168	0.45*	0.10	71634	0.23*	0.20	0.13	0.09
Condition2	8835	0.48*	0.11	8138	0.00†	0.20	0.18	0.11
Mortality1>2	0.1304	0.14*	0.06	1.303	0.14*	0.09	0.00	0.03

† Singularity problems in separating genetic and tank variance from each other.

Table 3. Phenotypic (above diagonal) and genetic (below diagonal) correlations between measured traits. Trait abbreviations are explained in Table 1. Significant correlations are bolded and marked with *.

	Diplo1	Cataract1	Weight1	Condition1	Weight2	Condition2	Mortality1>2
Diplo1		0.51	0.15	0.06	-0.05 (-0.10)†	-0.02 (-0.05)‡	0.06
Cataract1	0.79 ± 0.09*		0.16	0.06	-0.01 (-0.06)†	0.06 (-0.05)‡	0.00
Weight1	0.46 ± 0.16*	0.46 ± 0.17*		0.51	0.45	0.17	0.02
Condition1	0.27 ± 0.17	0.32 ± 0.16*	0.48 ±0.14*		0.03	0.39	0.16
Weight2	-0.21 ± 0.19 (-0.40 ± 0.18)†*	0.11 ± 0.19 (-0.07 ± 0.20)†	0.56 ± 0.17*	0.07 ± 0.17		0.43	-0.14
Condition2	-0.05 ± 0.20 (-0.22 ± 0.20)‡	0.15 ± 0.20 (-0.03 ± 0.21)‡	-0.00 ± 0.19	0.52 ± 0.14*	0.56 ± 0.13*		0.08
Mortality1>2	0.31 ± 0.21	-0.03 ± 0.20	-0.06 ± 0.24	0.65 ± 0.16*	-0.45 ± 0.16*	0.28 ± 0.19	

† Correlation estimated with a model in which Weight1 was used as a fixed covariate for Weight2

‡ Correlation estimated with a model in which Weight2 was used as a fixed covariate for Condition2

Table 4. Genetic variation in fish tolerance slopes (Vg_{slope} ±SE), the intercepts (Vg_{int} ± SE) and the genetic correlation between the slopes and intercepts (rg_{int_slope} ±SE). Trait abbreviations are explained in Table 1. Significant variances and genetic correlations are bolded and marked with *.

Trait against Diplo1	Vg_{slope}	±SE	Vg_{int}	±SE	$rg_{int\ vs\ slope}$	±SE
Weight1	1.702	2.683	466	91.2	-0.27	0.24
Condition1	0.054	0.416	93.7	24.3	-0.90	3.1‡
Weight2	194.2	1334	59965	19927	-0.99	2.5‡
Weight2†	206*	149	36716	20124	-0.99*	0.14
Condition2	303.2*	244	13545	3994	-0.52*	0.20
Mortality1>2	0.00206	0.003268	0.0308	0.0205	-0.67*	0.32

† Estimated with a model in which Weight1 was used as a fixed covariate for Weight2
‡ The model did not reach convergence

Table 5. Genetic correlations between resistance (number of Diplostomum sp. parasites) and tolerance (weight 1,2, condition 1,2, mortality 1>2). Trait abbreviations are explained in Table 1.

Trait whose tolerance slope is estimated	r_g: Tolerance vs Diplo1
$Weight1_{slope}$	0.03 ± 0.40
$Condition1_{slope}$	0.82 ± 3.25‡
$Weight2_{slope}$	-0.37 ± 1.58
$Weight2_{slope}$†	-0.12 ± 0.26
$Condition2_{slope}$	-0.18 ± 0.29
$Mortality1>2_{slope}$	0.21 ± 0.40

† Estimated with a model in which Weight1 was used as a fixed covariate for Weight2
‡ The model did not reach convergence